Effect of aggregation on morphine lethality in rats

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Chance (1946) first reported that placing amphetamine-injected mice into groups markedly reduced the LD 50. Since then the same phenomenon has been widely reported (e.g., Lasagna & McCann, 1957; Hohn & Lasagna, 1960; Wang, Hasegawa & others, 1969). All of these studies have demonstrated that the aggregation of mice potentiates the lethal effects of amphetamine.

Recently the phenomenon of group toxicity has been observed when aggregated mice were injected with opiates (Davis & Brister, 1971; Brister & Davis, 1974). These studies suggested that aggregation may also potentiate the lethal effects of morphine in mice. However the status of this suggestion is not clear, since Vedernikov (1970) has reported that aggregation did not effect morphine lethality in mice. To assess the role of aggregation in morphine lethality, we have investigated this phenomenon in rats.

160 male Wistar rats (Canadian Breeding Farms) 250–300 g were initially housed individually in stainless steel cages with Purina Lab Chow and water freely available.

In the first experiment 48 rats were injected intraperitoneally with 45 mg kg^{-1} of morphine sulphate and were either isolated in plywood boxes (8″ × 8″ × 10″), or aggregated in groups of 6 in large plastic baskets (12″ × 12″ × 10″). Twenty of the 24 grouped rats died after injection of this dose of morphine, whereas none of the 24 individually housed rats died. This difference was statistically significant (χ^2 (1) = 30·94, $P<0·01$).

We next attempted to demonstrate the group toxicity effect using a clearly non-lethal dose of morphine. Three groups of rats were injected with morphine sulphate (15 mg kg^{-1}, i.p.). The animals were either isolated in the plywood boxes, or aggregated in groups of 6 or 7 in the baskets. Again, as in the previous experiment, significantly more of the grouped rats died (χ^2 (2) = 64·99, $P<0·01$). Three of the 36 rats which were grouped 6 to a basket, and 12 of the 14 rats which were grouped 7 to a basket died, while none of the 50 isolated rats died. These results suggest that grouping potentiates the lethal effects of an otherwise non-lethal dose of morphine and that the degree of potentiation is a function of the size of the group.

Perhaps the most convincing demonstration of the morphine group toxicity effect in rats was obtained in the third experiment. In this experiment rats which had survived an intraperitoneal injection of a high dose of morphine in their home cages died when grouped and injected with a substantially lower dose several days later. Twelve isolated rats were injected with morphine (70 mg kg^{-1}, i.p.) in their home cages. None of these animals died. One week later 6 of the 12 rats were injected with 45 mg kg^{-1} of morphine individually in their home cages, while the other 6 rats were grouped in a basket and similarly treated. All of the 6 isolated rats survived, whereas 5 of the 6 aggregated rats died. This difference was significant (χ^2 (1) = 5·49, $P<0·05$) and demonstrates the impact of aggregation on morphine lethality.

The results of all three experiments indicate that grouping morphine-injected rats potentiates its lethal effects. The rats seemed to be dying of respiratory congestion. All exhibited wheezing sounds. Squeezing the chests of the dead animals produced a flow of fluid from their nostrils.

Group toxicity with amphetamine has been attributed to the synergism of the stimulant effects of amphetamine itself and the stimulation resulting from grouping the animals (Chance, 1946; Lasagna & McCann, 1957; Hohn & Lasagna, 1960; Wang & others, 1969). Although our animals did appear to be initially stimulated by the morphine, 20 min after injection the rats were motionless and seemed to be heavily sedated. This period of inactivity and sedation lasted approximately 2–2½ h. All of the deaths in the grouped animals occurred during this period, usually about 2 h after injection and suggest that, unlike amphetamine, the potentiation of the lethal effects of morphine by aggregation is not caused by a synergism between the stimulant effects of the morphine and the stimulation due to grouping.

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Lithium reduces preference for ethanol induced by hypothalamic stimulation

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Ho & Tsai (1975) have reported that treatment with lithium reduced the preference for dilute solutions of ethanol displayed by rats that had limited experience with ethanol. Lithium would be valuable clinically if it could reduce the consumption of ethanol by organisms with a history of persistent self-administration. We therefore decided to examine the effects of lithium on the chronic preference for concentrated solutions of ethanol established by hypothalamic stimulation in rats (Amit, Stern & Wise, 1970; Amit & Stern, 1971; Corcoran & Amit, 1974).

Eighteen adult male Wistar rats (Canadian Breeding Farms) with a monopolar stainless steel electrode implanted in the left lateral hypothalamus were housed individually in stainless steel cages with free access to food. Fluids were available in two calibrated glass Richter tubes (Kimax), containing either tap water or varying concentrations of 95 % ethanol diluted with tap water to form solutions of the desired concentration (v/v). The positions of the tubes containing water and ethanol were alternated to prevent the development of a position habit. The aversive cutoff concentration of ethanol was determined for each rat with the method of Amit & others (1970); these concentrations ranged from 10 to 19 % (v/v) in different rats, with a mean of 13·7 %. Following the procedure of Amit & others (1970), ethanol solutions were available on even-numbered days, with water available at all times. For the first 30 days of the experiment, ten of the rats received 30 min of daily hypothalamic stimulation according to Amit & Stern (1971). The schedule of stimulation was discontinued on day 31 and the ethanol intake of all rats was examined without further intervention until day 170. Beginning on day 171, a day in which only water was available, all rats received twice-daily intraperitoneal injections of lithium chloride at a dosage of 0·3 m equiv kg^{-1} (Ho & Tsai, 1975); according to Ho & Tsai, this dosage of lithium results in plasma concentrations of approximately 0·2 m equiv $litre^{-1}$. The injections were given at 12 h intervals. After 10 days (5 days of ethanol presentation), the dosage of lithium was raised to 0·6 mequiv kg^{-1}, which was administered for 6 additional days (3 ethanol days). To control for the possibility that the effects of lithium on ethanol consumption were due to the stress of the injections rather than to the lithium itself, a control group of 8 additional rats was prepared after the original experiment was completed. These rats had developed a preference for ethanol after a schedule of hypothalamic stimulation, and were treated in the same manner as above except that they received twice-daily injections of isotonic saline instead of lithium chloride. The data were analysed with one-way analysis of variance for repeated measures and, when post-hoc comparisons were justified, the Scheffe test (Ferguson, 1966).

For the purposes of this experiment, preference for ethanol was defined as intake greater than 65 % of total in a choice with water. At the time injections of lithium began, 10 of 10 rats subjected to hypothalamic stimulation had developed a consistent preference for ethanol, whereas 4 of 8 non-stimulated rats preferred ethanol to water. Administration of lithium produced a significant reduction ($P < 0.05$) in intake of ethanol, as shown in Fig. 1A and B. The reduction in ethanol intake was evident with the lower dosage of lithium (0·3 m equiv kg^{-1}), and subsequent doubling of the dosage of lithium not only failed to produce a further reduction in intake of ethanol, but actually seemed to result in a slight increase in intake. As can be seen in Fig. 1A, the reduction in intake of ethanol was not due to a nonspecific depression of drinking, because it was compensated for by a significant increase in intake of water. Repeated intraperitoneal injections of saline had no effect on the control group's intake of ethanol, indicating that the effects of the lithium injections were not due to the stress of the injections *per se*.

There were marked individual differences in the responses of the rats to lithium, in that the rats with a long-standing preference for ethanol were least affected by lithium, whereas a greater depression of intake was observed in the rats that did not prefer ethanol or had developed a preference only recently. For example, the 10 rats most affected by lithium (i.e., a significant reduction in intake of ethanol on at least 5 of the 8 drug sessions) had preferred ethanol for a mean of 20·6 of the 50 sessions preceding treatment with lithium, whereas the 8 least-affected rats had preferred ethanol

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